SPERMATOGENESIS OF THE DOG

BY

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The problem of spermatogenesis has been extensively worked on in insects and various other invertebrates. Recently several vertebrates have been shown to possess the so-called X-chromosome which is associated with sex-determination. Two hetero-chromosomes have been described in most of the mammals, and they probably correspond to the X- and Y-elements as described by Wilson ('12) in the Hemiptera. Different members of the latter class show a variation of these elements from forms which have only the X-element, thru those which have the X- and Y-element of unequal size, to those in which these elements are of equal size.

In the dog I find only one such element, the history of which I have tried to follow in this paper. The present study has been carried on at the suggestion of Professor M. F. Guyer, to whom I am especially indebted for kindly help and criticism. I wish also to acknowledge my obligation to Dr. Elizabeth A. Smith for valuable criticism.

MATERIALS AND METHODS

Thru the courtesy of the Department of Physiology of the University of Wisconsin, I was enabled to get the material in the living condition. Eleven of the animals from which the tissues were taken were mongrels; the twelfth was a thorobred bulldog. No especial differences were noted in the material except occasional minor discrepancies in the size of the cells.

At first samples from different regions of the living testes were placed directly into the fixing reagents in the preliminary preparations, but the best results were obtained when the tissues were allowed to stand for twenty minutes before being immersed in the fixative. As experienced by all other workers on mammals the great problem is to get the chromosomes to stand out as individual elements, the tendency being for them to mass-up before the fixative takes effect. For this reason the following fixatives: Gilson's, Carnoy's, Flemming's strong, Bouin's, Tower's, Herrmann's, Haner's modification of Flemming's strong, and Allen's modification of Bouin's, were tried out several times in an effort

to overcome this difficulty. It was found that the latter reagent gave the best results. As urea in this solution presumably increases the rate of penetration of the fixative, the amount of urea was varied from one to five percent in order to determine the optimum concentration for this tissue. A four percent solution was found to give the most satisfactory results. The formula for this fixative is as follows: to 100 cc. of Bouin's fluid add slowly 1.5 gm. of chromic acid and 4 gm. of urea just before using. Heat to 37° C., add the fresh material and leave it in the fixative for one to two hours keeping the temperature up to 37° C. For good cytoplasmic fixation put the fresh material in the fixative at 60° C. allow to cool to 37° C. and keep at this temperature as before. The fixative is then replaced gradually by 70 percent alcohol until entirely removed. A convenient apparatus for this part of the technic is described by Allen ('16). The tissues are then dehydrated, placed in xylol or chloroform, xylol-paraffin and imbedded in paraffin. As the material fixed better after standing 20 minutes the experiment was tried to see if allowing the tissues to autolyze from one to six hours would improve the fixation. It was found that there was no perceptable difference in the appearance of the nucleus altho the cytoplasm showed evidence of being digested and the chromosomes showed a tendency to clump up more after the first hour of autolysis.

Flemming's strong and Haner's modification of Fleming's strong gave good nuclear figures but the chromosomes did not stand out very clearly. Carnoy's fixative gave good spindle figures but always distorted the cells.

The sections were cut 5 microns and 7 microns thick and were stained with: (1) Haidenhain's iron-haemotoxylin and counter-stained with eosin, Bordeaux red, acid fuschin, or orange G; (2) Saffranin-lichtgrun; (3) Saffranin-gentian violet; (4) Benda's mitochondrial method. The most satisfactory stain was the iron-haemotoxylin counter-stained with acid fuschin, altho the saffranin preparations were valuable in studying the resting stages, different phases of the growth period, and the shape and number of the chromosomes.

Smear preparations were fixed in Allen's modification of Bouin's fluid and stained with Haidenhain's iron-haemotoxylin and Benda's mitochondrial stain. These preparations gave as good results in all stages as was found in the sections and were of advantage in so far as the cells were isolated, thus removing the danger of any confusion due the surrounding cells or to part of the cell being cut away.

Arrangement of the Germ Cells in the Testis

The main bulk of the testis is made up of the coiled seminiferous tubules which contain the germ cells. The seminiferous tubules are held together by connective tissue which contains a small number of interstitual cells, blood and lymph vessels. Practically all stages of development of the germ cells may be found in a single tubule. In general the spermatogonia and the Sertoli cells are at the periphery, then comes the primary spermatocytes, the secondary spermatocytes, the spermatids and the spermatozoa. There is no definite seriation of the stages in any one tubule, such as is seen for example, in the insect testes. Some tubules are filled practically with cells in the growth stages, others with spermatids and some with nothing but a few Sertoli cells and fibers left by the discharged spermatozoa.

It is thus apparant that the great problem in Mammalian spermatogenesis is to determine the seriation of the stages of development. The criterion employed was, mainly, resemblance to the stages in forms which had already been worked out. The size of the different cells and the number of chromosomes were the chief guides.

Spermatogenesis

Spermatogonia

The spermatogonia usually lie at the periphery of the tubules, but occasionally when rapid multiplication is taking place, they are found in the deeper portions. The spermatogonia are hard to distinguish from the Sertoli cells but usually they can be identified by the small amount of their cytoplasm. The nuclei of the Sertoli cells present the same general appearance as those of the resting spermatogonia and as far as I can determine are the same. According to some investigators they both have a common origin from the primordial germ cells (Hegner '14).

There are present in the resting spermatogonial nuclei from one to four large, deeply staining nucleoli which always fuse when activity commences. This process of fusion is seen best in the saffranin preparations, which show that these bodies approach each other but before coming in contact seem to be connected by thin threads of chromatin on each side. They then completely fuse forming an oval nucleolus (figs. 1-3). Even in the earliest periods when these bodies are well separated they seem to be connected by thin fibers or strands of chromatin. A number

of interlacing linin threads along which small granules of chromatin are deposited radiate from the nucleolus (fig. 4). As activity commences in the nucleus there is a marked increase of chromatin granules about the nucleolus as though they were threading out from it (fig. 5). At the same time the amount of chromatin along the linin fibers increases until the linin is practically obscured from view. Undoubtedly some of this chromatin along the threads is from the nucleolus but the increase is so great that some must either be synthesized or is present in the resting nucleus in such a chemical structure that it does not react to the stains used The cytoplasm which is made up of short interlacing fibers contains in all stages a spermatosphere. This stains more intensely with cytoplasmic stains.

As development continues the separate threads of chromatin shorten and become thicker. These finally condense into the chromosomes of the spermatogonial metaphase, as shown in figs. 7 and 8. It appears as though a single thread does not become a single chromosome but that parts of threads condense about different "centers" which are connected to each other by the linin fibers. The nucleolus does not lose its identity thruout these stages but remains a large, dark body with threads radiating from it. It does, however, approach the chromosomes in size in the early prophase (fig. 7), but it soon becomes more darkly stained than they (fig. 8). Cells showing the nucleolus as in fig. 7 are very few hence it indicates that this stage is of very short duration. This so-called nucleolus is probably the X-chromosome as it can be traced as such throuout all the later stages by its staining reaction. The newly formed chromosomes now take up their usual position on the spindle as twenty-one oval-shaped bodies of which one is the X-chromosome. Information as to the origin of spindle fibers and of the centrosomes was not obtainable but they were very distinct in all cases. During the formation of the spindle, the nuclear wall disintegrates. The spermatogonial metaphase is probably of short duration as stages where none of the chromosome had started to divide, as in fig. 9, were very few. Counts were made from polar views (Fig. 10), and oblique side views of the metaphase spindles. In material obtained from a female foetus an attempt was made to determine the somatic count in tissues taken from the pancreas, liver, mesonephros, metanephros and kidney. A few clear spindles were secured from liver material in which 22 chromosomes appeared (fig. 69).

In cases where the autosomes had just started to divide (figs. 11, 13, 14), it was obvious that the X-chromosome was dividing ahead of them.

In the middle anaphase (figs. 15 and 16) the X-chromosome can be seen still slightly ahead of the autosomes, which are now clumping together. The early telophase (fig. 17) shows the chromosomes clumped with a spermatosphere near each mass. The cell wall (fig. 18) constricts in the middle of the long axis and finally divides the cell into two complete daughter cells. The spindle fibers are still present but soon disappear leaving the cells with a mass of chromatin, and the spermatosphere imbedded in the cytoplasm. Thus the cells are ready for the growth period as primary spermatocytes.

Growth Period

Stage A-preleptotene (figs. 19 and 20). In this stage the cells from the spermatogonial telophase have the chromosomes clumped together in an irregular granular mass. The nuclear membrane has not yet formed. This stage possibly represents that described by Wilson ('12) in *Oncopeltus* and *Lygaeus* as an uncoiling of the individual chromosomes to form separate leptotene threads, but as the chromosomes are so massed the actual processes cannot be determined. A large black body which is present in this mass of chromatin retains its identity throughout the growth period and from its subsequent behavior will be called the X-chromosome. Fig. 20 shows the leptotene threads emerging from the chromatin mass.

Stage B-leptotene (fig. 18). The nuclear wall is now present and is seen to enclose several thin, beaded threads of chromatin and the X-chromosome. These leptotene threads do not form a continuous spireme but appear as independent threads in both the smear and section preparations. While the threads show no definite polarization such as Wenrick ('16) found in the *Phrynotettix magnus*, they form a network which makes them very hard to count. They have not been seen to exceed twenty in number which further indicates that they take their origin in the spermatogonial telophase chromosomes.

Stage C-synapsis and synizesis (fig. 22). The leptotene threads of stage B drift toward one pole of the nucleus where they condense into a mass. The parts of the nucleus not occupied by these threads is clear. This stage has been studied in several forms of mammals by von Hoff ('12) who concludes that synizesis is the result of the action of the fixative but as pointed out by Wilson ('12), Fasten ('14) and others it occurs in the living material and thus cannot be the result of the action

of the fixative. In cases of poor fixation, however, the leptotene threads are so contracted that their individuality cannot be made out, while with proper fixation it is very clear that the threads do not lose their identity during this contraction. They appear to arrange themselves in pairs for they emerge from the mass in parallel strands.

Stage D-pachytene (fig. 23). The leptotene threads which paired up in the previous stage now fuse side by side; that is, undergo parasynapsis. This is conclusively shown in this figure as in many others where two leptotene threads can be seen fused at one end and separated at the other. The line of fusion of these threads is not obliterated until a much later stage. Thus the dog is another form which shows parasynapsis such as described by von Winniwarter ('09) Gregoire ('04), Schreiners ('04), Wilson ('12) Smith ('16), and others.

Stage E-diplotene (fig. 24). This stage is hard to distinguish from stage D except that all the leptotene threads have fused to form the thicker diplotene threads. In these the line of fusion of the leptotene threads cannot be seen except in well destained preparations. The ends of the threads appear thicker than the rest of the thread. This stage might be called the beaded stage for each thread has the appearance of a string of beads. They approximate the haploid number. It will be noted that thruout these stages the X-chromosome does not lose its identity and that the spermatosphere is present.

These diplotene threads now contract gradually into somewhat oval-shaped chromosomes. As this contraction progresses, linin threads connecting them appear. In the late prophase the nuclear wall breaks down and the chromosomes take their places upon the primary spermatocyte spindle. Since twenty-one chromosomes entered the primary spermatocyte from the spermatogonial division, the leptotene threads paired and the X-chromosome did not lose its identity, it is obvious that the spermatocyte autosomes must be bivalent; that is, each one is made up of two univalent chromosomes, and the X-chromosome is univalent. This material contained no indications that the leptotene threads twisted about each other to form chiasmas such as observed by Janssens ('09) and Smith ('16). Heterotypic tetrad figures in the late prophase stages are not apparent although preparations stained favorably with iron-haemotoxylin reveal a quadrapartite appearance of the bivalent chromosomes as tho they were preparing for the following maturation divisions.

The actual growth period might be considered to be from stage C to E as there is very little change in volume up to the time of synizesis but from there on the increase is very marked. The diplotene stage probably lasts longer than any other as cells in this stage of development are found in large numbers in the majority of the tubules.

Reduction Division

When the primary spermatocytes are ready for division they reveal ten large bivalent chromosomes, and one large X-chromosome. As the X-chromosome lies, in the metaphase, in very close proximity to the autosomes it is often difficult to determine its shape. But it can be distinguished from the others as a longer, slightly curved body, (figs. 28, 29, 32, 35, 38, 39) similar to that noted by Guger ('12) and ('16). However, a curved body from the concave or convex side would appear as an oval body (fig. 30 and 34). Fig. 31, a polar view, presents clearly the reduced number of chromosomes. All the chromosomes of the equatorial plate were not usually in the same plane.

As division starts in the autosomes the X-element can be seen to pass slightly ahead of them to one pole. The dividing chromosomes are long and thin giving the appearance of overlapping each other (fig. 36 and 37). This division is probably the reduction division as the autosomes divide longitudinally and the X-chromosome passes unchanged to one pole. In fig. 41 there could be counted at one pole, ten ordinary chromosomes plus the accessory while only ten autosomes passed to the other pole. It will also be noted that the spermatosphere is still present.

Interkinesis

After the primary spermatocyte division no resting stage occurs. The secondary spermatocyte metaphase is formed by the rearrangement of the chromosomes present in the primary spermatocyte telophase.

In the late anaphase of the primary spermatocyte the centrioles with short spindle fibers between them and the chromosome masses (fig. 44) are apparent, indicating that the chromosomes do not reach the poles as in the other divisions. The division of the centrioles and the formation of the new astral system cannot be followed but from the appearances of such cells as figs. 46 and 47 one might infer that there is not a new astral system formed but that the remnants of the previous spindle are re-organized to form the secondary spermatocyte spindle. The

chromatin mass is imbedded in a more or less clear space surrounded by cytoplasm.

As two types of cells enter this stage two kinds must result from their division, one with ten univalent autosomes, (fig. 49), and one with ten univalent autosomes plus the X-chromosome, (fig. 48). When the autosomes divide they pull apart in the center, the X-chromosome dividing slightly ahead of them and passing to each pole, fig. 52. In the telophase of this division the chromosomes are usually massed up but in such a figure as 54 approximately ten chromosomes plus the accessory can be distinguished passing to each pole whereas in others no trace of an accessory can be found, fig. 55. Thus two kinds of spermatids which will develop into mature sperm result from this division.

Spermiogenesis

The chromosomes of the second maturation division break up into an open reticulum composed of linin threads and chromatin granules. The nucleus thus goes into a resting condition which apparently lasts for some time. In approximately half of the nuclei there can be seen a definite round body, fig. 60, which possibly corresponds with the X-chromosome It can be seen in the nucleus after condensation of the chromatin has occurred and the nucleus has migrated to one side of the cell, fig. 62 and 63.

The spermatosphere, which takes the cytoplasmic stains, is present in all of the spermatids in the cytoplasm and either imbedded in it or closely associated with it can be seen the centrosome. In the same region of the cytoplasm, fig. 57, is found the idiozome or remnant of the previous spindle.

The centrosome and the idiozome are differentiated from one another by the fact that the centrosome remains in close apposition to the spermatosphere. It is a single, regular body while the idiozome is usually lobular and irregular. As the idiozome comes in contact with the nuclear wall an oval, clear space as described by Leplat ('10) in the cat appears between it and the nuclear wall (fig. 58). It is apparently caused by some repulsive force between the nuclear membrane and the idiozome for the nuclear wall is definitely depressed. The wall of the cavity opposite the nuclear wall is probably formed by material from the idiozome. The nuclear wall then gradually returns to its original position, fig. 60, the nucleus becoming longer than it is wide and the idiozome

forming a cap over about two-thirds of its length. Later this cap becomes the acrosome of the mature sperm fitting closely over the anterior end of the head. There seems to be little change in the idiozome threads during this transformation but the dark mass at the tip disappears. In sections threads running between the Sertoli cells and the acrosome were usually noted but no indication of their origin could be found unless they come from the acrosome. Fig. 68 shows a tubule from which all of the sperm have been discharged and has nothing in it but a few Sertoli cells with these threads running to them.

While this development of the acrosome has been going on the spermatosphere and the centrosome have migrated to the opposite side of the nucleus. The spermatosphere becomes closely applied to the nuclear wall and the centrosome divides into an anterior and a posterior portion, fig. 61. At this stage the nucleus gets smaller and the chromatin material appears as an indefinite granular mass in which a dark body, possibly the remnant of the X-chromosome, is seen in approximately half of the cells, fig. 62. The nucleus migrates to the side of the cell toward the acrosome apparently carrying with it a definite amount of cytoplasm enclosed in a denser wall, fig. 62. The migration of the nucleus is usually toward the tubule wall. At the base of this cytoplasmic neck which is destined to become the sheath of the middle piece of the mature spermatozoa, is found the spermatosphere. It retains this position until the nucleus, now the sperm head, has broken thru the cell wall. In the "giant cells" (fig. 74) in which a number of spermatid nuclei are present in one cell, there is a spermatosphere associated with each nucleus.

The process of formation of the acrosome and middle piece is similar to that found by McGregor ('99) in *Amphiuma*, except that he finds part of the centrosphere or idiozome also going to form the middle piece.

After division of the centrosome the anterior one comes in contact with the nuclear wall causing a temporary depression in the latter and then flattens out into a disc between the walls of the cytoplasmic neck. This disc, which forms the end knob of the sperm, has extending back from it a thin filament which extends to the spermatosphere fig. 63. Attached to this filament by a fine stalk is the posterior centrosome. This condition differs from that described by Leplat ('10) in the cat and Wodsedalek ('13) in the pig, in that the posterior centrosome in these animals forms a ring which migrates along the axial filament and is cast off with the cytoplasm.

As the sperm head breaks thru the cell wall the axial filament becomes much longer. The flattened head is composed of three regions of different staining reaction, fig. 64. The point of attachment of the acrosome corresponds with about the posterior margin of the anterior two-thirds of the head and the point of attachment of the cytoplasmic neck corresponds with the anterior margin of the posterior third of the head. Thus it appears that the difference in the density is due to the presence of these membranes and that the lighter middle portion is due to the absence of these membranes. Further evidence of this is seen in fig. 65 in which the acrosome is becoming applied to the nuclear wall. Here there are only two regions of different color. This observation may be carried to the mature sperm, fig. 67, which shows in a side view that there is a layer of dark staining material covering the posterior third of the head, which also appears darker than the rest of the head when viewed from above, fig. 66.

When the cells reach the stage shown in fig. 65 they become attached in groups to the Sertoli or nurse cells by long filaments. At this time the cytoplasm is cast off and the sperm continue to develop. The surrounding cytoplasm is found to disappear gradually and as the sperm do not leave the tubule until this is about complete, it is possible that they derive some of their nourishment from this bed of cytoplasm. In the Benda preparation there was found large and small globules of fatty substance in this cytoplasm, in the Sertoli cells and a few in the interstitual cells. These globules stain black with iron-haemotoxylin but do not stand out as well as in the Benda preparations.

In the final changes of the sperm the acrosome becomes closely applied to the sperm head and is no longer distinguishable. The cytoplasm of the middle piece contracts and slightly elongates to cover almost the anterior half of the sperm tail or axial filament. During this contraction the posterior centrosome breaks thru the cytoplasmic wall and is seen lying outside the middle piece (fig. 66). There is no evidence of the sperm head enlarging during this process as described by Wadsedalek ('13).

Thus the mature sperm is seen to consist of: a head formed from the entire nucleus of the spermatid, the cytoplasm of the middle piece from the spermatosphere and the axial filament together with the anterior and posterior centrosomes from the centrosomes. The spermatozoa now lose their attachment to the Sertoli cells and pass into the lumen of

the tubule leaving behind the Sertoli cells with large bundles of threads attached to them as shown in fig. 68.

Further evidence of the dimorphism of the sperm was obtained by camera lucida measurements. Three hundred measurements were made at 2,000 magnification of mature sperm, obtained from the vas deferens. It was found that these sperm, selected at random from the preparation, could be grouped into two classes on the basis of size. The heads of one hundred and sixty-one at this magnification measured five to six millimeters when projected onto paper with a camera lucida while the balance of the three hundred measured seven to eight millimeters. This difference in size is probably due to the presence of the X-chromosome.

Summary

1. The five typical cells ordinarily found in spermatogenesis: spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids, and sperm occur in the dog in an unseriated arrangement. These are enclosed in long, thin, winding tubules which are held together by connective tissue and interstitutal cells.

2. Large numbers of spermatogonia and Sertoli cells are present around the periphery of the tubules and may contain one or more large, deeply staining bodies or nucleoli. In case of the spermatogonia these nucleoli always fuse before activity is marked. This body is possibly associated with or is the X-chromosome.

3. The spermatogonia show twenty-one oval shaped chromosomes, the X-chromosome usually not being distinguishable until the early anaphase where it divides and passes to the poles slightly ahead of the autosomes.

4. Following the spermatogonial division the chromosomes weave out into separate leptotene threads, while the X-chromosome remains as a rounded or slightly oval dark-staining mass.

5. The leptotene threads undergo parasynapsis.

6. Eleven chromosomes appear in the primary spermatocyte, ten are bivalent autosomes and one the X-chromosome. The X-chromosome passes undivided to one pole while the autosomes divide by longitudinal splitting. Thus there are produced two kinds of secondary spermatocytes. This division is reductional.

7. There is no resting stage between the primary and secondary spermatocyte divisions, the chromosomes retaining their identity although they increase in size slightly.

8. The two kinds of secondary spermatocytes upon division give rise to two kinds of spermatids, one with ten univalent autosomes and the other with ten univalent autosomes plus the X-chromosome. This dimorphism is further evidenced by the resting spermatids as approximately half of them show a large, deep staining body which is probably the X-chromosome.

9. The chromatin of the spermatid nucleus condenses into an indifferent mass, the nucleus contracts, becomes narrower and flattened. It passes to one pole of the cell, breaks thru the cell wall and leaves most of the cytoplasm of the cell behind. It then attaches itself to a Sertoli cell by a thin fiber and shapes up into a mature sperm.

10. During spermiogenesis the centrosome gives rise to the end knob, axial filament and the posterior centrosome; the sphere substance of the secondary spermatocyte division to the acrosome; and the spermatosphere to the sheath of the middle piece.

11. Measurements of mature sperm give a distinct bimodal curve, also indicating their dimorphism.

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EXPLANATION OF PLATES

All drawings were made at 1600 magnification with a camera lucida. The plates were reduced about one-fourth.

Plate IX

Figs. 1, 2 and 3. Resting spermatogonial nuclei.

Fig. 4. Resting spermatogonia.

Figs. 5 and 6. Spermatogonia in early stage of activity showing the increase in chromatin along the linin fibers.

Figs. 7 and 8. Spermatogonia showing the condensation of the chromatin to form the chromosomes of the metaphase. Fig. 8 is from a smear preparation.

Fig. 9. Side view of a spermatogonial metaphase.

Fig. 10. Polar view of a spermatogonial metaphase showing 21 chromosomes.

Figs. 11, 12, 13 and 14. Early spermatogonial anaphase showing the chromosomes starting to divide.

Figs. 15 and 16. Spermatogonial anaphase showing the X-chromosome dividing ahead of the autosomes.

Fig. 17. Early telophase of the spermatogonial division.

Fig. 18. Late telophase of the spermatogonial division.

Figs. 19 and 20. Preleptotene stage or beginning growth period. The dark staining compact mass is the body which later is seen to be the X-chromosome.

Fig. 21. Leptotene stage showing the X-chromosome.

Fig. 22. Synapsis and synizesis showing the drifting of the leptotene threads and their contraction to one side of the nucleus.

Fig. 23. Pachytene stage showing the chromsomes pairing by parasynapsis.

Figs. 24 and 25. Diplotene stage showing the beaded appearance and the unfused threads.

Figs. 26 and 27. Late primary spermatocyte prophase showing the condensation of the diplotene threads to form the chromosomes.

Figs. 28, 29, 30, 32, 33 and 35. Side views of primary spermatocyte metaphase, figs. 28, 29, 32 and 35 showing the curved X-chromosome.

Fig. 31. Polar view of a primary spermatocyte metaphase showing ten autosomes and the X-chromosome.

Figs. 34, 36, and 37. Early primary spermatocyte anaphase showing longitudinal division of the autosomes.

Figs. 38 and 39. Parts of primary spermatocyte anaphases showing the curved X-chromosome going to one pole.

Figs. 40 and 41. Early primary spermatocyte telophase.

Figs. 42, 43 and 44. Late primary spermatocyte telophase.

Figs. 45, 46 and 47. Secondary spermatocyte prophase.

Figs. 48 and 50. Secondary spermatocyte metaphase showing ten autosomes plus the X-chromosome. Fig. 50 from a smear preparation.

Fig. 49. Secondary spermatocyte metaphase showing ten autosomes.

Fig. 51. Early secondary spermatocyte anaphase showing equational division. From a smear preparation.

PLATE X

Figs. 52 and 53. Early secondary spermatocyte anaphase showing equational division.

Fig. 54. Secondary spermatocyte telophase showing approximately ten autosomes plus the X-chromosome at each pole.

Fig. 55. Same as fig. 54 except that the X-chromosome is not present.

Figs. 56 and 57. Spermatid showing the spermatosphere with the centrosome imbedded in it and the remnants of the secondary spermatocyte spindle (idiozome).

Figs. 58, 59, 60 and 61. Spermatids showing the formation of the acrosome from the idiozome, the migration of the spermatosphere plus the centrosome to the other side of the nucleus, and the division of the centrosome.

Figs. 62, 63 and 64. Spermatids showing the migration of the nucleus to one side of the cell, the formation of the middle piece, end knob, posterior centrosome and the axial filament.

Fig 65. Immature sperm which has cast off its cytoplasm except that destined to become the sheath of the middle piece and the fibre which seems to connect it with the Sertoli cell.

Fig. 66. Mature sperm viewed from the broad side.

Fig. 67. Mature sperm viewed from the side.

Fig. 68. Cross section of a tubule the sperm cells of which have all matured leaving only a few Sertoli cells and fibres.

Fig. 69. Polar view of a somatic cell of a female foetus showing 22 chromosomes.

Figs. 70 and 71. Anomalies showing heterogenic division.

Figs. 72 and 74. Giant cells showing more than one nucleus and their associated spermatospheres.

Fig. 73. Two spermatids side by side.

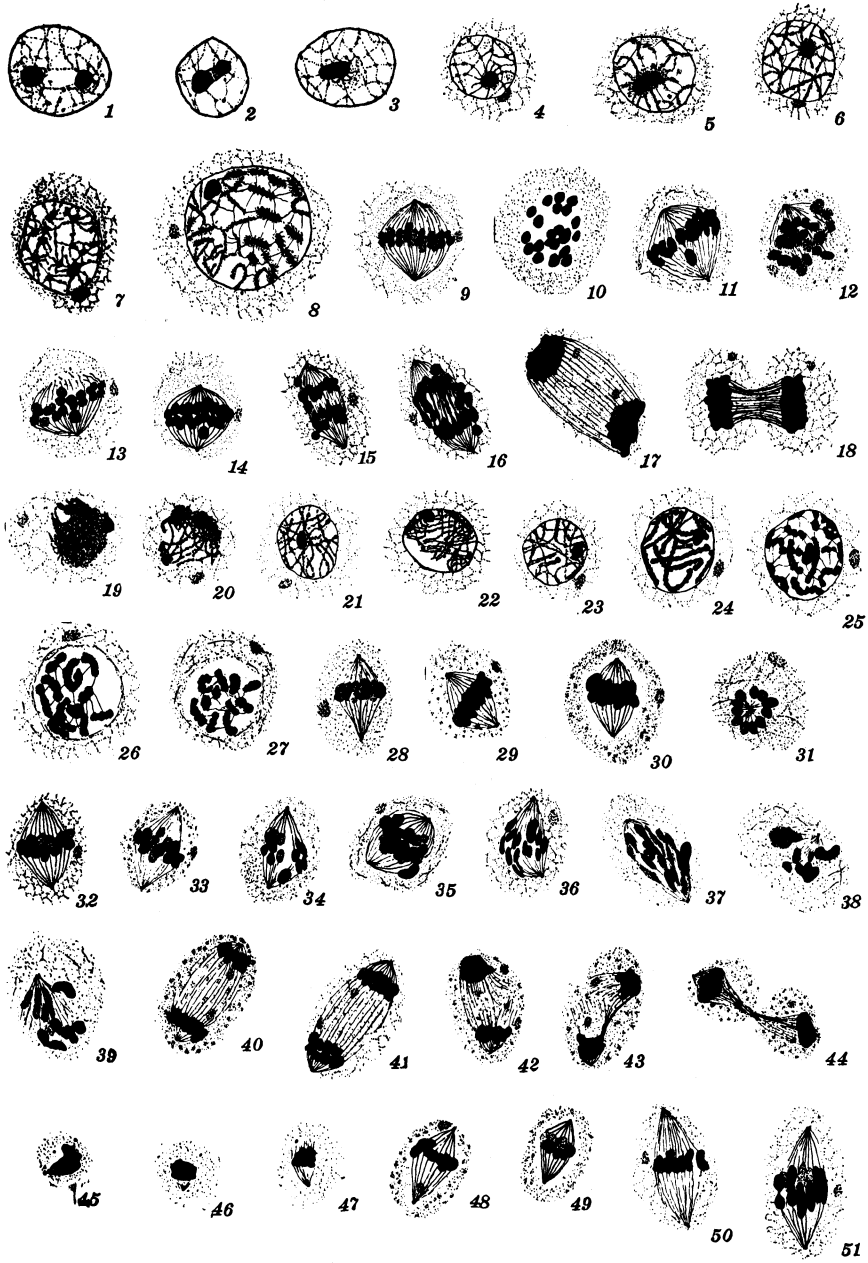

PLATE IX

MALONE

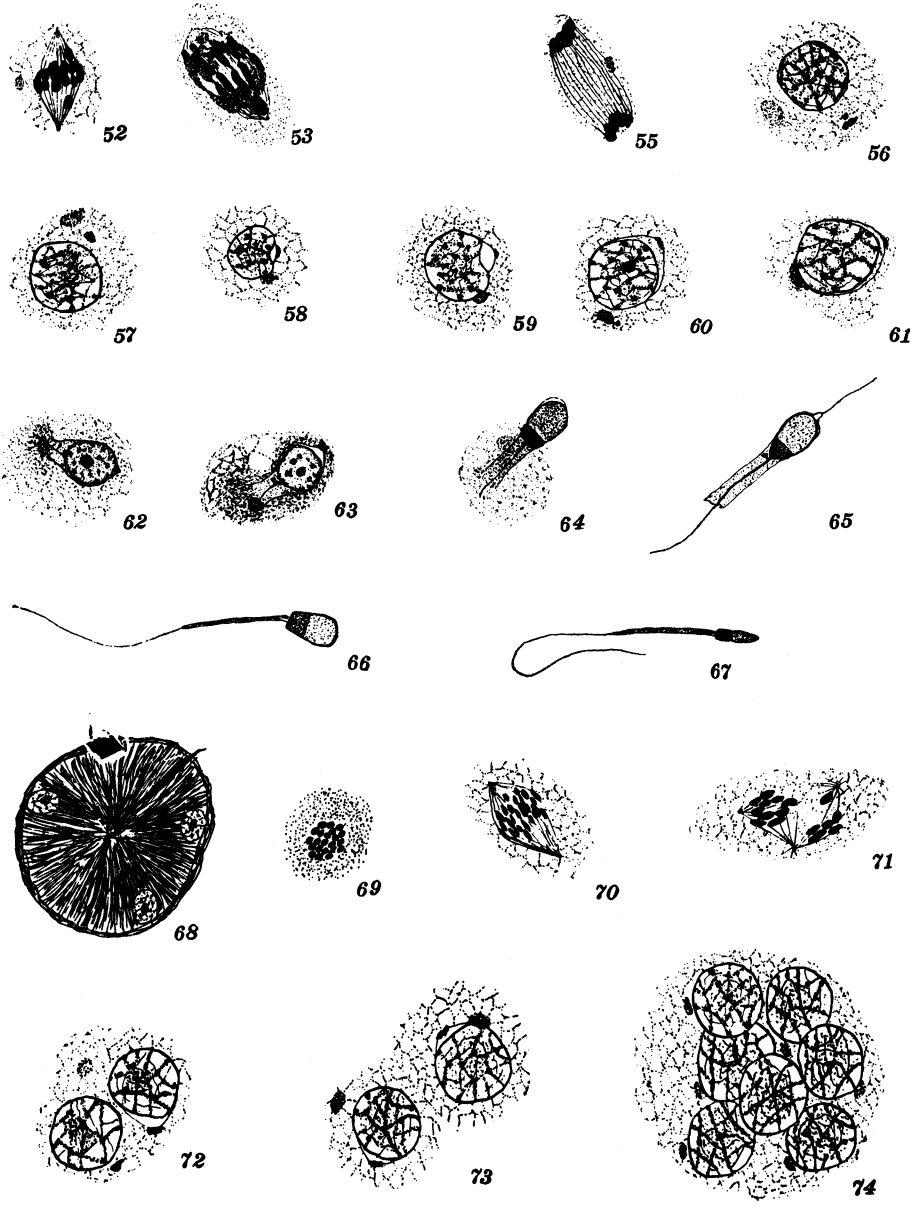

PLATE X